and a cytoplasmic region. The extracellular region has five tandemly repeated ectodomains (EC1-EC5), with three calcium binding sites situated between each of these domains. Cell-cell adhesion is mediated by the dimerization of cadherins presented on neighboring cell surfaces. We are focusing our studies on two members of type I classical cadherins, Neural (N) - and Epithelial (E)-cadherins. In spite of high sequence similarity between E- and N-cadherin, they have distinct physiological localization implying differences in their adhesive properties. Here, we compare the spectral characteristics, stability, calcium binding and assembly properties of the first two domains of N- and E- cadherins. Spectroscopic studies of these proteins were predictable and indicated typical β-sheet conformation with only partial exposure of tryptophans. Although both proteins are stabilized by calcium, apo-ECAD12 is less stable than apo-NCAD12. Direct calcium titrations that found the proteins bind calcium with equally high affinity (−6.2 kcal/mol). There was a striking difference between these proteins in terms of the kinetics of disassembly. Analytical size exclusion chromatography experiments showed that disassembly of ECAD12 dimers is rapid and disassembly of NCAD12 dimers is slow regardless of the calcium concentration. We observe this striking difference with constructs containing only the first 220 residues of a 700 residue protein. Thus, not only is this an interesting protein folding-function question, this remarkable difference in these cadherins may explain their segregation into different physiological niches.

2312-Pos

Effect of Osmolytes on Proteins

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Cells exhibit cellular coping mechanisms when faced with osmotic stresses by importing or producing compounds called osmolytes, which aid in osmotic regulation. Proline is an example of such a compound. The primary function of these compounds is to combat the effects of dehydration in the cell. Stabilization of proteins, which are susceptible to osmotic stresses, is of key importance to the cell's health. Osmolytes have been shown to impact the stability and solubility of proteins, and certain osmolytes also exhibit the function of aiding in protein folding and refolding and in preventing protein aggregation. The mode by which osmolytes aid in protein stabilization is believed to be a solvent-oriented process by which folding is facilitated by the preferential ordering of solvent molecules, but the exact mechanism remains elusive. We characterized the supramolecular structure of proline in solution using multi-dimensional NMR spectroscopy and dynamic light scattering. The molecular mechanism underlying the stabilizing effect of proline on a protein is studied using thermal denaturation monitored by steady-state fluorescence. Results from the denaturation studies indicate that the Tm of the protein increases in the presence of increasing concentrations of proline by about 20 °C, suggesting that thermodynamic stability of the protein is enhanced upon binding to proline. Stability studies using several other osmolytes like TMAO, glycerol, 4-hydroxy proline, and betaine show that proline is the osmolyte which stabilizes the protein to the largest extent. Two-dimensional HSQC NMR experiments were used to reveal the proline binding sites on FGF-1. The results provide useful insights on the molecular mechanism of proline. The mechanism by which proline stabilizes protein is further investigated in hydrogen-deuterium exchange experiments monitored by NMR with the protein in the presence and absence of proline in viscous medium. These results provide valuable thermodynamic and binding specificity data.

2313-Pos

How Stable is an Enzyme from a Thermophilic Organism? Denaturation Studies with the Esterase from *Pyrococcus furiosus* - The Role of Charge-Charge Interactions

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We have expressed the gene of the hyperthermophilic esterase (Pf2001Δ60) from *Pyrococcus furiosus.* This esterase showed to be active after boiling and has a half-life of 120 min at 75 °C. We decided to study the unfolding of this enzyme by fluorescence spectroscopy induced by urea, guanidinium hydrochloride (GndHCl) and high hydrostatic pressure (HHP). Ours results pointed out that urea is about three times more efficient than GndHCl in promoting structural perturbations in PF2001Δ60 ([D1/2]=7.1 and 2.3 M, respectively), suggesting that ion pairs are important stabilizing factors on its structure. There was almost no change in the tryptophan center of mass of PF2001Δ60 (342 nm) under HHP up to 3.1 kbar, even by staying 120 min under 2,500 bar. Interestingly, the combination of HHP with a subdenaturating concentration of urea (1 M) displaced the center of mass by ≈ 7 nm after 40 min at 2,500 bar. Since HHP enhances the electrostriction, this result reinforces the crucial contribution of salt bridges in esterase's stability. The binding of the bis(8-anilinonaphthalene-1-sulfonate) to PF2001Δ60 was increased by 2.5- and 3-fold after treatment with 2 M GndHCl or under HHP combined with urea, suggesting that these treatments convert the enzyme into a partially folded intermediate with exposed hydrophobic regions. Altogether, ours results may be an indication that the optimization of charge-charge interactions on the protein surface is a key factor for its stability. To our knowledge, this is the first time that HHP is used to access the ion pair contributions to the stability of a hyperthermophilic esterase.

2314-Pos

Cysteine Reactivity as a Probe of the Proteome

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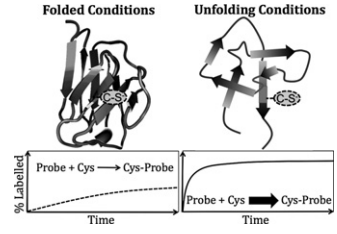
The study of protein folding and stability is typically conducted with purified proteins by methods that are exacting but lack the ability to analyze complex mixtures of proteins. To study proteins in their native environments inside a cell or isolated organelle, such as a nucleus, we have recently developed a powerful method that combines shotgun labelling with LC/MS/MS. Cysteine is a reactive but hydrophobic amino acid that can be fluorescently labelled in isolated nuclei with time resolution under stress conditions (changes in temperature). Quantitative kinetic analyses of spectra allow us to identify regions in hundreds of proteins, including nuclear lamins implicated in diseases such as Progeria, to understand the folding and interactions in situ. Select protein domains are also studied in solution, demonstrating the close correspondence to more traditional methods.



2315-Pos

Nanomechanics of Ankyrin-R Repeats Probed by AFM and SMD Simulations

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Ankyrin (ANK) repeats, identified in thousands of proteins, are composed of pairs of antiparallel alpha-helices that stack on top of each other and form super-helical spiral domains with suggestive spring-like properties, whose primary function is to mediate specific protein-protein interactions. For example, ankyrin-R links the anion exchanger in the erythrocyte membrane to the membrane skeleton and contains 24 ANK repeats that form a spiral domain. Ankyrin-R stabilizes the erythrocyte membrane and mutations in ANK repeats are documented in hereditary spherocytosis (HS), the life-threatening human anemia. Since repeats 13 to 24 (D34) of Ankyrin R are especially active in binding interactions and are subjected to the HS mutations, therefore the mechanical properties of this region of ankyrin-R are of the utmost importance. Although the conserved residues in each ankyrin repeat generate nearly identical helix-helix-loop structures, the exact positions and numbers of internal hydrogen-bonds, salt bridges and hydrophobic residue packing between repeats vary along the D34 domain and this variation is expected to modulate the elasticity, mechanical stability and mechanical unfolding/refolding properties of D34. We probed these properties of D34 directly by means of AFM-based single molecule force spectroscopy and by Steered Molecular Dynamics (SMD) simulations. By mapping the AFM force spectroscopy data onto the SMD-determined behavior of the internal network of H-bonds and salts bridges, we propose a model of the complex mechanical unfolding and refolding patterns of ANK repeats in D34.

2316-Pos

Cation-Pi Interactions Contribute Significantly to the Stability of FGF and the FGFR

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Fibroblast growth factors (FGFs) are ~ 16 kDa heparin binding proteins that regulate key cellular processes such as angiogenesis, differentiation, morphogenesis, wound healing and tumor growth. FGFRs consist of three extracellular ligand binding domains (D1, D2, D3), a single transmembrane helix, and cytoplasmic tyrosine kinase domain. Cell surface-bound HSPGs (heparan